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Scientific Note

OCCURRENCE OF A NEOGREGARINE PROTOZOAN, *OPHRYOCYSTIS ELEKTROSCIRRHA* MCLAUGHLIN AND MYERS, IN POPULATIONS OF MONARCH AND QUEEN BUTTERFLIES

The monarch butterfly, *Danaus plexippus* (L.), along with another New World species, the queen butterfly (*Danaus gilippus berenice* Cramer) are both susceptible to a neogregarine parasite, *Ophryocystis elektroscirrha* McLaughlin & Myers, in Florida. The neogregarine parasite, described by McLaughin & Meyers (1970. J. Protozool., 17: 300–305), involves a life cycle strategy that incorporates the occurrence of spores on the scales and hairs of the infected butterflies. The neogregarine has been reported from monarch butterflies in California (Leong, K. et al. 1992. Ecol. Entomol., 17: 338–342), Hawaii and Mexico (Brower, L. et al. 1995. BioSci., 45: 540–544). However, except for California, the prevalence of infection and the occurrence of this neogregarine in other populations of the monarch butterfly and a closely related species, the queen butterfly, are unknown. Accordingly, we conducted a survey of monarch butterflies from various parts of the world and queen butterflies from Florida to gain a better insight into their distribution and prevalence of infection.

Our survey confirms the presence of *O. elektroscirrha* on monarch butterfly adults collected from Hawaii, Mexico, Florida, Australia and New Zealand (Table 1). The survey data show that the percentage of butterflies with spores (based on a wash method by Leong et al. 1992) ranged from 3.8% (Mexico) to 100% (Maui, Hawaii). Among butterflies with spores, those collected from Oahu, Hawaii had the highest average number of spores per individual (197,480) and those from New Zealand, the least (10,500). Six of the 32 queen butterflies (*D. gilippus berenice*) examined had neogregarine spores (18.8%) with an average of 1500 spores per individual.

The spore dimensions varied considerably depending upon the geographical regions and species (Table 2). The queen butterflies collected from Fort Lauderdale, Florida had the smallest spores whereas monarch butterflies from California, Hawaii and the queen from Gainesville, Florida, had the largest. Notably, spores from the queen butterflies had both the smallest (Fort Lauderdale) and largest (Gainesville) measurements. Spores recovered from butterflies collected in Fort Lauderdale were significantly smaller than those recovered from queen butterflies collected in Gainesville or from monarch butterflies ($P < 0.01$, $F = 12.8$, $df = 7,313$; Table 2). The different sizes within the queen butterflies suggest the occurrence of distinct strains or possibly another neogregarine species, perhaps one unique to the queen.

The larger neogregarine spores isolated from *D. gilippus berenice* from Gainesville, Florida, were infectious to the monarch butterfly. When leaves of the blood flower milkweed, *Asclepias curassavica* L., were sprayed with 50,000 spores/ml until runoff and fed to first instars, 3 of 9 (33.3%) monarch larvae became infected. We were unable to test the pathogenicity of the smaller neogregarine spores

Table 1. Isolation of neogregarine spores from monarch butterflies (*Danaus plexippus* [L.]) from various geographical areas and from the queen butterfly (*D. gilippus berenice* Cramer) from Florida.

. Species/Location	*n*	#S/*n*[a]	(%)	Mean[b]	(Range)[c]	M/F[d]
Monarch butterfly						
United States of America						
Florida						
Broward County	7	6/7	(85.7%)	347,200	(200–232,000)	6/1
California						
San Luis Obispo County	160	92/160	(57.5%)	59,470	(200–565,000)	80/80
Santa Cruz County	130	86/130	(66.2%)	76,000	(400–497,000)	70/60
Hawaii						
Oahu County	17	14/17	(82.3%)	197,480	(200–974,000)	11/6
Maui County	2	2/2	(100%)	17,900	(2000–33,800)	1/1
Kauai County	3	2/3	(66.7%)	47,600	(2000–94,800)	2/1
Hawaii County	7	5/7	(71.4%)	42,600	(400–240,000)	6/1
Australia	20	12/20	(60%)	53,400	(200–436,000)	10/10
New Zealand	7	1/7	(14.3%)	300	(300)	3/4
Mexico	26	1/26	(3.8%)	1400	(1400)	10/16
Queen butterfly						
Florida (combined)	32	6/32	(18.8%)	1500	(200–8600)	23/9
Gainesville	14	2/14	(14.3%)	600	(200–1000)	10/4
Fort Lauderdale	18	4/18	(22.2%)	2400	(200–8600)	13/5

[a] = no. of individuals with spores/total number of individuals.
[b] = average spore level from abdomens of butterflies.
[c] = range of spore levels among individuals with spores.
[d] = male/female.

from Fort Lauderdale to monarchs because they were recovered earlier in our investigation (January 1993), and a colony of protozoan-free monarch butterflies was not available at that time.

Neogregarine spores recovered from the monarch butterflies were not infectious to the silkworm, *Bombyx mori* (L.). When 30 first instar silkworm larvae were

Table 2. The length, diameter and area of neogregarine spores recovered from monarch and queen butterflies from various geographical areas. Spore area with different superscript letters are significantly different ($P < 0.01$, $F = 12.8$; $df = 7313$).

Species/Location	*n*	Length (um)	Diameter (um)	Area (um^2)
Queen, Fort Lauderdale, Florida, USA	37	12.3 ± 0.2	8.1 ± 0.2	101.2 ± 2.6[a]
Monarch, Australia	50	13.9 ± 0.1	7.8 ± 0.3	114.0 ± 1.7[b]
Monarch, New Zealand	22	13.0 ± 0.2	8.9 ± 0.2	117.2 ± 3.2[bc]
Monarch, Fort Lauderdale, Florida, USA	50	14.1 ± 0.1	8.4 ± 0.1	118.2 ± 1.2[bc]
Monarch, Mexican	42	13.9 ± 0.1	8.6 ± 0.2	119.6 ± 2.9[bc]
Monarch, California, USA	50	13.7 ± 0.1	8.8 ± 0.1	120.6 ± 2.2[c]
Monarch, Hawaii, USA	50	14.3 ± 0.1	8.9 ± 0.1	127.3 ± 1.9[c]
Queen, Gainesville, USA	30	13.2 ± 0.2	9.6 ± 0.1	127.4 ± 2.6[c]

fed mulberry leaves sprayed with 100,000 spores/ml until run-off, none of the resulting adults had neogregarine spores.

Thirty-five striated queen butterflies, *D. g. strigosus* (Bates), a subspecies found in the desert regions of Colorado and southern California, were examined for neogregarine spores. None of the adults surveyed had neogregarine spores. Their absence in the striated queen population may reflect an inadequate sample size, infection levels too low to be detected with our method, or the resistance of this subspecies to the protozoan. A more likely explanation is that the striated queen is susceptible to the protozoan, but are not exposed to the parasite because they do not share a common milkweed host plant with the monarch butterfly. The striated queen larvae feed mainly on the rambling milkweed, *Sarcostemma hirtellum* R. Holm, a plant not used for oviposition by monarch butterflies. The populations of queen and monarch butterflies of south central Florida are ecological competitors during early spring (March-May). The adults of the two species are found in the same habitat where they will feed on similar nectar sources and oviposit on common *Asclepias* plants. The larvae of the two species have been reported on the same milkweed host (Brower, L. 1961. Ecol., 42: 76–83).

Our previous observations indicated that the neogregarine is passed vertically from one generation to another by infected adults contaminating eggs or milkweed leaf surfaces with spores directly or with scales containing spores during oviposition. The larvae become infected when they ingest the spores. Our survey data show that the infection level of butterflies overwintering in California ranges from 200 to > 900,000 spores per individual. Even at the higher levels of infection in the California monarch butterfly populations, the protozoan appeared to have little effect on the butterfly's winter survival and mating successes (unpublished data). The low pathogenicity of this parasite on its host in nature has allowed it to persist widely within populations of monarch butterflies. Studies on the genus *Danaus* suggest that the monarch butterfly evolved in the New World, probably South or Central America (Kitching, I. et al. 1993 pp. 11–16. *In* Biology and conservation of the monarch butterfly, Natural History Museum of Los Angeles County, Los Angeles, CA). We hypothesize that the neogregarine co-evolved with its host and because of the high rate of infection, the parasite moved with its host into other geographic regions. We cannot discount the possibility that the queen butterfly or other related Danaid species were the original host for this neogregarine which subsequently became adapted to the monarch butterfly. Regardless, both the queen and monarch butterfly are hosts of *O. elektroscirrha*. Examination for neogregarine spores on other closely related species to the queen and monarch butterflies may provide further insights into the distribution of *O. elektroscirrha* and its host spectrum.

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